ASSESSMENT OF SOME AGRONOMIC AND SEED QUALITY TRAITS IN *BRASSICA CARINATA* LANDRACE GENOTYPES, DOUBLED HAPLOID LINES AND HYBRIDS

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Abstract

The present study was undertaken to evaluate some important agronomic traits (yield, 1000 seed weight, plant height, oil content and fatty acid composition) and crossability of B. carinata and B. napus in the green house. Remarkable changes in oil (32.49%) and erucic acid (20%) content were recorded in crossing of DH BC Dodolla with DH BN SL-730. Satisfactory combining ability of several DH lines of BC resulted in heterosis effect in obtained hybrids. Hybrids between DH lines of BC 6 with DH line BC Dodolla showed intermediate height of their parents. The DH line Dodolla proved to be the most productive DH regenerant in our experiments. Therefore interspecific and intraspecific hybridization in Brassicas can lead to wider utilization of Brassica carinata (Ethiopian mustard) in food and feed industry.

Key words: *Brassica carinata, Brassica napus* , interspecific hybrids, seed oil quality

INTRODUCTION

Ethiopian mustard (*Brassica carinata* A. Braun) has long been known to be one of the oldest crops in the plateaus of east Africa (Gomez-Campo and Prakash, 1999). The crop has good agronomic traits like high yielding, better resistance to disease, insect pests and seed shattering than any one of the oilseed crops adapted to comparable areas (Nigussie et al., 2001), with the additional agronomic advantage of its better tolerance to semiarid conditions However, it possesses high erucic acid in the oil and high glucosinolate content in the meal. Recently, researchers in Canada, Spain and India showed interest to this crop due to its tolerance to biotic and abiotic stress under semi-arid conditions (Rakow, 1995).

Analysis of genetic relationships in crop species is an important component of crop improvement. It helps to analyse genetic variability of cultivars select parental materials for hybridisation for making new genetic recombinations, select inbred parents or tester for maximum heterotic response and identify materials that should be maintained to preserve maximum genetic diversity in germplasm sources (Adefris and Becker, 2005).

Efforts have been made to develop low-erucic acid genotypes of *B. carinata* utilizing different strategies such as inter-specific crossing with *B. napus* (Fernandez-Escobar et al., 1988, or with *B. juncea* (Getinet et al., 1994), induction of mutations (Velasco et al., 1995a) or continued crossing and pedigree selection within the *B. carinata* germplasm (Alonso et al., 1990).

Main objectives of the present study were to assess and compare some agriculturally important traits in selected landrace genotypes, doubled haploid lines and hybrids of *B. carinata* and partially *B. napus* genotypes with the possibility to identify perspective plant materials, utilisable in consecutive breeding programmes.

MATERIALS AND METHODS

Plant materials

Two high productive doubled haploid (DH) lines of winter oilseed rape (*Brassica napus* L.) (BN) C-612 from Breeding Station Chlumec nad Cidlinou, Selgen, Inc. and SL-730 from Breeding Station Slapy u Tábora, Sempra Praha, Inc., one commercial cultivar Labrador, four genotypes BC 1, BC 2, BC 4, BC 6 and one cultivar Dodolla of Ethiopian mustard (*Brassica carinata* A. Braun) and subsequently derived DH lines (Eyasu et al., 2007) and hybrids, were used for experiments.

Hybridization

Young flower buds of appropriate size of female parents, supposed to be open in the next morning, were emasculated and other opened flowers and immature buds were removed. The emasculated buds were then pollinated with fresh pollen collected from male parents and were covered immediately with bags from unwoven cloth to protect the pollinated stigma for about 5–7 days.

Recording of data for agronomic characters

Data for different characters, i.e. plant height (5 plants in each genotype), number of pods per plant, number of seeds per pod, weight of seeds per plant, 1 000 seed-weight (three plants in each genotype), oil content and fatty acid content (assessed from the populations, donor plants, DH regenerants and hybrids) were recorded.

Determination of seed oil quality

Single fatty acid (FA) assessment in seeds of oilseed rape was carried out in Research Institute of Oilseed Crops in Opava according to the ‘Model of single fatty acid assessment in rapeseed oil by the method of Gas Chromatography (GC)’’ modified for the analysis of single seed sample (Kolovrat, 1985). Methylesters of fatty acids were analysed on gas chromatograph CHROM 5 with writer type TZ4200, using glass column (2.5 mm length, 3 mm inside diameter) with the refill: 3% SP-2310/2% SP-2300 at 100/120 Chromosorb WAW (SUPELCO). Temperature program was used in the analysing process.

RESULTS

In total, 854 emasculated flowers were pollinated with pollen collected from selected parents. While the success of the hybridization within genotypes of *B. carinata* was relatively high (from 76.2% to 90.8%), interspecific hybridization with *B. napus* genotypes was less efficient (Table 1), both in case of the *B. carinata* mother parent and reciprocal crossings (from 18.6 to 27.0%). Moreover, the number of seeds per pod obtained from intraspecific hybridization experiments (9.4–14.4) was nearly six times higher than in case of interspecific hybridization with *B. napus* (1.6–2.7). Better results were obtained from crossings with *B. napus* as a moth- er, where the number of seeds per pod varied from 4.2 to 6.4 (Table 1). Seed colour of selected genotypes and resulting hybrids is documented in Figure 6.

According to statistical analysis, the effect of a genotype on the plant height was significant. Measured plant height of tested *B. carinata* DH plants and hybrids varied significantly from 1 200 mm in the DH line of the genotype BC 6 to 1 466 mm in the DH line of genotype Dodolla (Table 2, Figure 3). Both hybrids between selected DH lines (Dodolla x BC 4 and Dodolla x BC 6) achieved intermediate height of their parents (1 356 mm and 1 296 mm). Additionally, mean heights of DH Dodolla and DH BC 6 genotypes were significantly different both from each other and from all other DH lines and hybrids tested (Table 2, Figure 3).

The impact of a genotype on the number of pods per plant was relevant. The lowest mean number of pods per plant was obtained in the DH line BC 6 (250.3); significantly higher numbers of pods were observed in DH lines BC 1 (310.3), Dodolla (360.0) and in F1 hybrids between DH Dodolla and DH BC 6 (339.7) and between DH Dodolla and BC 4 (295.0). Moreover, the number of pods in the DH line Dodolla was significantly higher than in other genotypes tested, except one F1 hybrid (Table 3, Figure 4).

Tab. 1: Crossability within genotypes of *Brassica carinata* and *Brassica napus*

Crossing results / Crossing combinations	No. of flowers pollinated	No. of pods developed	No. of developed pods with seeds	% of success	Total no. of seeds obtained	No. of seeds per pollination	No. of seeds per pod
	a	b	c	(c/a)x100	d	d/a	d/b
DH BC Dodolla x DH BC 1	87	80	75	86.2	750	8.60	9.4
DH BC Dodolla x DH BC 2	65	61	59	90.8	855	13.10	14.0
DH BC Dodolla x DH BC 4	102	89	82	80.4	1025	10.00	11.5
DH BC Dodolla x DH BC 6	91	85	71	78.0	958	10.50	11.3
(DH BC Dodolla x DH BC 4) x DH BC Dodolla	59	51	49	83.1	735	12.45	14.4
(DH BC Dodolla x DH BC 6) x DH BC Dodolla	42	37	32	76.2	416	9.90	11.2
DH BC Dodolla x DH BN SL-730	82	36	17	20.7	59	0.72	1.6
Reciprocal	39	15	9	23.1	63	1.60	4.2
DH BC Dodolla x DH BN OP-612	97	31	18	18.6	67	0.69	2.2
Reciprocal	43	18	11	25.6	88	2.00	4.9
DH BC Dodolla x BN Labrador	89	35	24	27.0	94	1.00	2.7
Reciprocal	58	22	15	25.9	142	2.44	6.4
Total – Cross	**714**	**505**	**427**	**59.8**	**4959**	**6.90**	**9.8**
Total – Reciprocal	**140**	**55**	**35**	**25.0**	**293**	**2.10**	**5.3**

Tab. 2: Plant height (in millimetres) in selected DH lines and hybrids of *B. carinata*

Genotype	Replication					Mean	SD	Groups
	1	2	3	4	5			
DH Dodolla	1480	1420	1450	1480	1500	1466	31.30495	a
DH BC 1	1400	1410	1380	1400	1370	1392	16.43168	b
DH BC 4	1400	1320	1400	1420	1250	1358	71.55418	bc
DH Dodolla x DH BC 4	1380	1350	1330	1370	1350	1356	19.49359	bc
DH BC 2	1350	1320	1270	1300	1320	1312	29.49576	c
DH Dodolla x DH BC 6	1320	1350	1200	1360	1250	1296	68.77500	c
DH BC 6	1140	1220	1300	1140	1200	1200	66.33250	d

Letters a–d designate homogeneous groups (LSD; $P = 0.05$) derived from multiple comparisons between means
SD – Standard Deviation

Tab. 3: Number of pods per plant in selected DH lines and hybrids of *B. carinata*

Genotype	Replication			Mean	SD	Groups
	1	2	3			
DH Dodolla	405	325	350	360.00	40.92676	a
DH Dodolla x DH BC 6	355	320	344	339.67	17.89786	ab
DH BC 1	300	337	294	310.33	23.28805	bc
DH Dodolla x DH BC 4	315	292	278	295.00	18.68154	c
DH BC 2	284	291	265	280.00	13.45362	cd
DH BC 4	270	282	274	275.33	6.11010	cd
DH BC 6	259	247	245	250.33	7.57188	d

Letters a–d designate homogeneous groups (LSD; $P = 0.05$) derived from multiple comparisons between means
SD – Standard Deviation

Tab. 4: Seed yield (in grams) per plant in selected DH lines and hybrids of *B. carinata*

Genotype	Replication			Mean	SD	Groups
	1	2	3			
DH Dodolla x DH BC 6	45.40	39.80	44.30	43.17	2.96704	a
DH Dodolla x DH BC 4	36.80	34.30	35.00	35.37	1.28970	b
DH Dodolla	32.80	29.50	31.80	31.37	1.69214	c
DH BC 6	31.50	28.30	29.70	29.83	1.60416	c
DH BC 1	25.00	26.30	23.60	24.97	1.35031	d
DH BC 4	23.10	18.50	20.00	20.53	2.34592	e
DH BC 2	20.10	20.50	19.80	20.13	0.35119	e

Letters a–e designate homogeneous groups (LSD; $P = 0.05$) derived from multiple comparisons between means
SD – Standard Deviation

Fundamental differences were detected between genotypes of *B. carinata* in case of the seed yield per plant. Generally, heterosis effect was confirmed in our experiments. Both hybrids (DH Dodolla x DH BC 6 and DH Dodolla x DH BC 4) were significantly more productive than all DH lines tested (Table 4, Figure 5). The less productive DH lines, genotypes BC 2 and BC 4, yielded only 20.1 and 20.5 g of seed per plant, while the yield in the best F1 hybrid was more than double (43.2 g per plant). Best DH lines, Dodolla and BC 6, produced 31.4 and 29.8 g of seed per plant.

The results showed only slight differences between 1 000 seed weight (Table 5) of DH lines (3.8–5.6 g) and correspondent original genotypes (4.7–5.7 g). In general, results obtained from hybrid plants showed greater differences in 1 000 seed weight compared to DH lines and populations as well. The highest rates were obtained in hybrids (BC 6 x Dodolla) x Dodolla and

Tab. 5: 1 000 seed weight, oil content and fatty acid composition in the seed of selected genotypes of *B. carinata* and *B. napus* planted in the glasshouse

Genotype	1000 seed weight [g]	Oil content [%] in dry matter	Palmitic 16:0	Stearic 18:0	Oleic 18:1	Linolic 18:2	Linolenic 18:3	Arachic 20:0	Eicosenoic 20:1	Eicosadienic 20:2	Behenic 22:0	Erucic 22:1	Docosadienic 22:2
			Faty acid content in oil [%]										
Population													
BC 1	4.71	35.76	2.7	0.7	9.3	19.3	14.2	0.6	6.2	0.8	0.5	44.2	1.2
BC 2	4.97	26.34	2.8	0.9	10.5	19.3	18.6	0.8	6.3	0.9	0.7	38.0	1.2
BC 4	5.22	28.21	3.4	0.7	5.7	14.4	17.7	0.7	6.7	0.8	0.6	47.4	1.8
BC 6	4.64	27.85	2.7	0.9	8.9	19.7	13.7	0.8	5.6	0.8	0.8	44.6	1.5
BC Dodolla	5.29	30.98	2.8	1.0	10.4	14.4	11.2	0.9	10.5	0.9	0.5	46.4	1.1
BC Dodolla (field condition)	5.66	54.70	3.1	0.7	11.8	15.1	14.6	0.8	7.4	1.0	0.6	43.4	1.6
Doubled haploid line													
DH BC 1	3.76	20.70	3.1	0.7	8.7	17.4	15.5	0.8	6.4	1.0	0.6	44.2	1.7
DH BC 2	4.48	20.24	2.9	0.9	9.7	20.0	11.3	1.1	5.7	0.9	1.0	44.4	2.0
DH BC 4	5.60	25.40	2.4	0.6	10.3	17.0	14.7	0.7	6.5	1.0	0.6	44.3	1.8
DH BC 6	4.43	26.10	2.6	0.7	9.3	19.4	14.5	0.7	6.5	1.1	0.6	43.1	1.6
DH BC Dodolla	5.19	25.29	4.1	0.6	8.6	18.4	17.3	0.7	6.5	1.0	0.4	40.7	1.7
DH BN SL-730	5.32	37.38	5.3	1.1	68.5	16.5	6.0	0.7	1.2	0.0	0.3	0.2	0.0
Hybridization													
DH BC 4 x DH BC Dodolla	6.51	29.80	2.6	0.7	11.6	18.1	15.0	0.7	6.3	0.9	0.7	41.9	1.5
DH BC 1 x DH BC Dodolla	5.12	32.26	2.6	0.7	10.3	17.1	14.9	0.6	6.9	1.0	0.6	43.7	1.7
DH BC 6 x DH BC Dodolla	4.92	29.46	3.0	0.8	10.5	19.9	12.3	0.7	6.3	1.0	0.7	43.0	1.6
DH BC 2 x DH BC Dodolla	4.83	24.66	2.9	0.8	10.8	18.0	13.0	0.7	7.5	1.0	0.7	43.1	1.5
(DH BC 6 x DH BC Dod.) x DH BC Dod.	6.96	30.88	2.6	0.6	8.6	16.7	15.5	1.1	7.1	1.1	0.6	44.1	2.0
DH Dodolla x DH BN SL-730	4.85	32.49	3.6	0.9	36.8	18.9	12.2	1.0	3.9	0.5	0.4	20.8	0.9

DH – doubled haploid line
BC – *Brassica carinata*
BN – *Brassica napus*

BC 4 x Dodolla (7.0 g and 6.5 g), the smallest in the DH line BC 1 (3.8 g). One *B. napus* genotype, DH line SL-730, displayed moderate rate 5.3 g (Figure 1).

Oil content in the dry matter of seeds, harvested from plants maintained in the glasshouse, was the highest in the *B. napus* DH line SL-730 (37.4%) and in the *B. carinata* genotype BC 1 (35.8%). Good results were obtained also in genotype Dodolla (31.0%) and in almost all crosses with the DH line Dodolla (Table 5, Figure 2). Worse results were obtained in case of all *B. carinata* DH lines. The lowest oil content was determined in genotypes DH BC 2 (20.2%) and DH BC 1 (20.7%).

Fatty acid composition of oil in selected genotypes is presented in the Table 5. Only slight or no differences in the content of individual fatty acids were observed in all tested genotypes of *B. carinata* (including populations, derived DH lines and hybrids). Characteristic composition of *B. carinata* and *B. napus* oil, represented by genotypes DH BC Dodolla and DH BN SL-730, is shown in Figure 7. The same figure and the Table 5 shows dramatic changes of the fatty acid composition after interspecific hybridization between *B. carinata* (female) and *B. napus*. Resulting hybrid has approximately half content of erucic, eicosenoic, eicosadienic and docosadienic acid than the mother genotype (*B. carinata*, DH line Dodolla); marked decrease can be seen in the content of linolenic acid as well. On the other hand, more than four times higher content was observed in case of oleic acid (36.8%, was 8.6%).

DISCUSSION

Good results obtained from hybridizations within *B. carinata* landrace genotypes, where about 80% of pollinated flowers formed pods, are caused most likely by the genetic relationship of genotypes used. Thus, no genetic barriers, which are often seen during intergeneric and interspecific hybridization experiments, were observed. Similar results were described by Malek et al. (2006) in interspecific hybridization between *B. carinata* and *B. rapa*. In contrast, crossings with unrelated genotypes of *B. napus* showed far worse results, where only approx. one fifth of pollinated flowers created pods with seeds. Also Choudhary et al. (2000) in *Brassica* interspecific crossing detected outstanding decrease in the number of developing pods in comparison with intraspecific hybridization. Moreover, the number of seed per pod was unsatisfactory in case of interspecific hybridization with *B. napus* as well, when only about two seeds per pod were observed. Conformable results were presented by Malek et al. (2006) in interspecific hybridization between *Brassica carinata* and *Brassica rapa*, where the number of seeds per pod varied from one to ten. Slightly better results were recorded when *B. napus* genotypes were used as mother components and pollinated with *B. carinata*. In this case the number of seeds per pod was almost half (about five seeds) of numbers obtained from intraspecific hybridization within *B. carinata* genotypes (about 11 seeds per pod). Positive effect of reciprocal crossings was also reported in cross between *B. rapa* and *B.carinata* by Malek et al. (2006).

Significant effect of a genotype on the plant height, number of pods per plant and the seed yield per plant was confirmed in our experiments and were already recorded for example in interspecific hybridization of *B.carinata* and *B. napus* by Bechyně (1992). From the set of genotypes tested, contrast DH lines could be selected. For instance, one DH line of the genotype BC 6 was only 1 200 mm high, while one DH line of the genotype Dodolla more than 1 460 mm. Hybrids between above mentioned lines showed intermediate heights of their parents. The DH line Dodolla proved to be the most productive in our experiments with the yield greater than 40 grams per plant, most likely due to its height and the number of pods per plant. Good combining ability of some tested DH lines, represented by heterosis effect, was confirmed in our experiments. Both hybrids were significantly more productive than the better of parents. Although there were visible differences in oil content between materials tested and contrast genotypes with high and low rates could be selected, overall percentage rate of oil content was rather low. For instance, one tested DH line of *B. napus*, SL-730, recorded only about 37 % of oil in the dry matter of seed, whereas common value for *B. napus* is usually about 60% and about 37–45% in *B. carinata* genotypes. These poor results could be most likely caused by different conditions in the field and in the glasshouse, where the plants were maintained till the harvest. For example, genotype Dodolla maintained in the glasshouse recorded only about 31% of oil in the dry matter. The same genotype produced almost 55% of oil in the seed, when planted and finally harvested in the field.

In spite of previously mentioned results, from our experiments can be seen markedly lower oil content in all DH lines in comparison with initial genotypes and hybrids. Concordant findings were made by Anke et al. (2007) in *B. napus* as well. This might be caused by some stress effects, acting within regeneration processes of doubled haploid plants and thus negatively affect physiological condition of regenerants and even maturing plants, or it might be evoked by the genotypic background of obtained DH regenerants.

No important differences were observed in the fatty acid composition between original landrace genotypes, DH lines and their hybrids of *B. carinata.* Contents of individual major and minor fatty acids in tested genotypes corresponded with the previous observations in genotypes of *B. carinata*. Huge changes were recorded after hybridization with *B. napus* especially in the content of erucic and oleic acid as the fatty acid composition of both parents is very different. Similar results were provided by Choudhary et al. (2000); Malek et al. (2006) in interspecific hybridization between *B. carinata* and *B. rapa*.

CONCLUSION

Some important agricultural traits were assessed in various genotypes of *B. carinata.* Although hybridization experiments within related genotypes showed good results both in the number of pods per plant and seeds per pod, the same values concerning interspecific hybridizations were unsatisfactory. To obtain better results in genotypes used, *B. napus* used as a mother component.

Significant impact of a genotype on some important agronomical characteristics (plant height, number of pods per plant, number of seeds per pod and the seed yield) was statistically verified. Satisfactory combining ability of several DH lines resulted in heterosis effect in obtained hybrids. Contrast DH lines in all above mentioned traits can be selected as perspective initial materials utilizable in consecutive breeding procedures.

Although marked differences between genotypes were detected in case of oil content, the percentages observed were considerably lower than those for corresponding *B. carinata* and *B. napus* genotypes previously published. This might be caused by different conditions between the glasshouse and the field. To obtain more accurate results, seeds for oil content analysis should be collected from plants maintained in the field. In spite of these results, lower content of oil in all DH lines in comparison with original genotypes and hybrids was evident and might be among others caused by the different physiological state of DH regenerants, derived from microspore embryos. Thus, it can be recommended to evaluate DH lines subsequently multiplied from seeds, not direct DH regenerants.

The fatty acid composition of tested *B. carinata* materials did not differ much between individual genotypes. Striking change in the content was recorded after hybridization with *B. napus* and thus can be utilized in breeding programs aimed at the improvement of Ethiopian mustard and lead to wider use of that important crop in food and feed industry.

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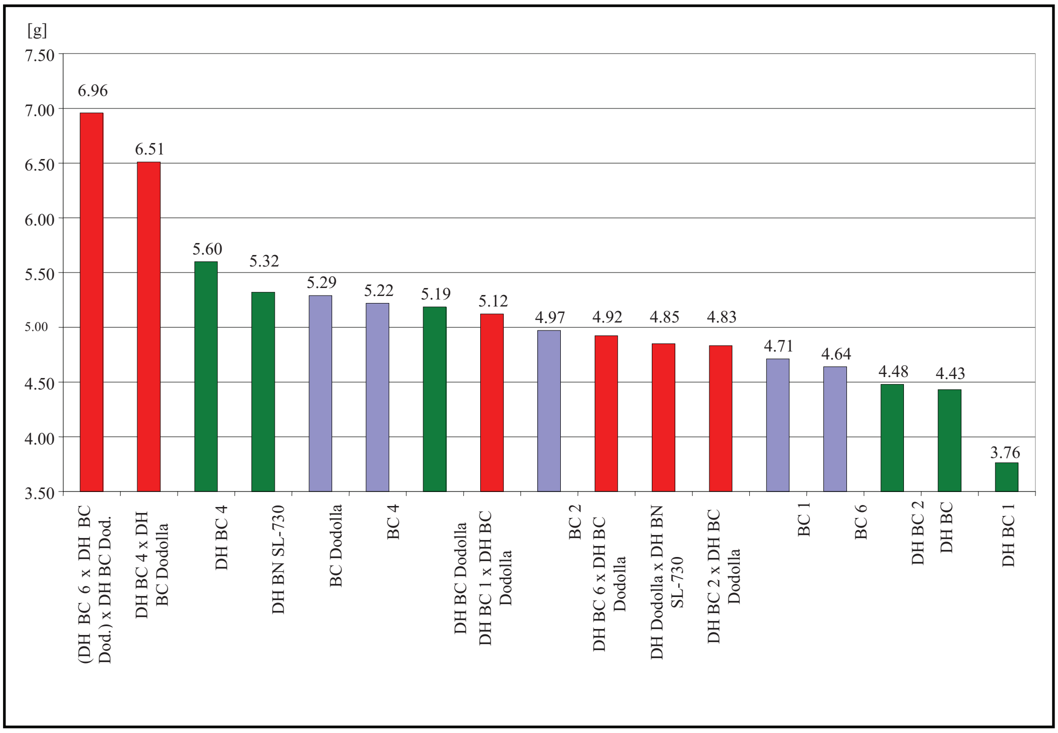
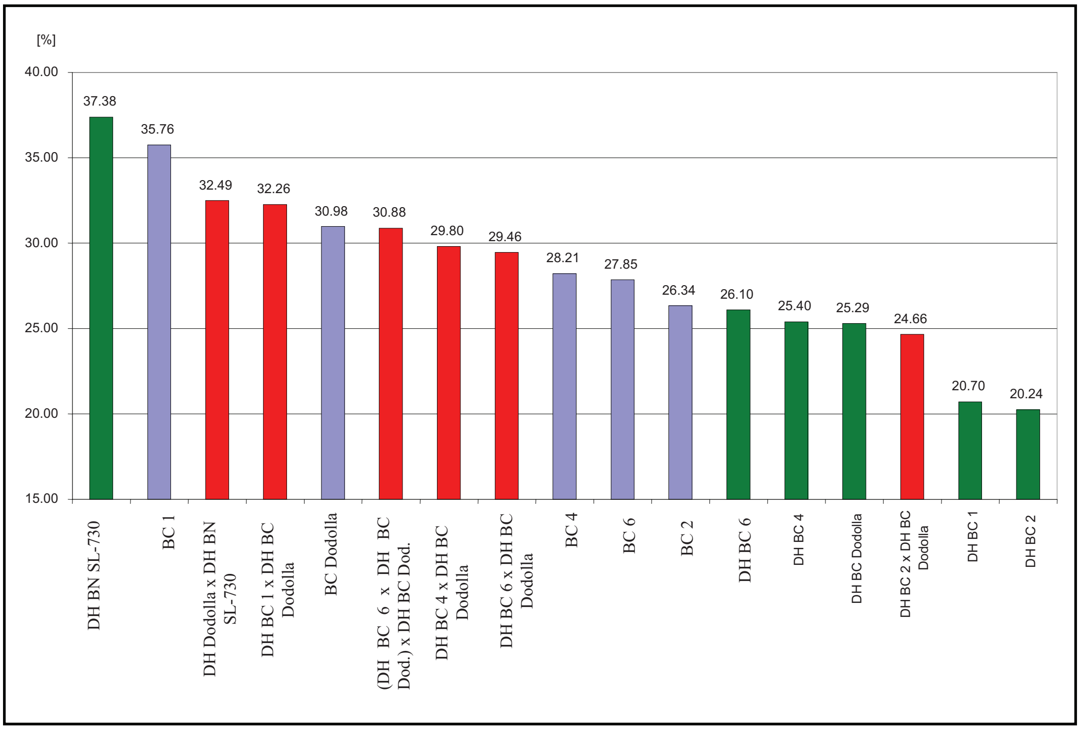
FIGURES

Figure 1: 1000 seed weight in selected genotypes of *B. carinata, B. napus* and their hybrids



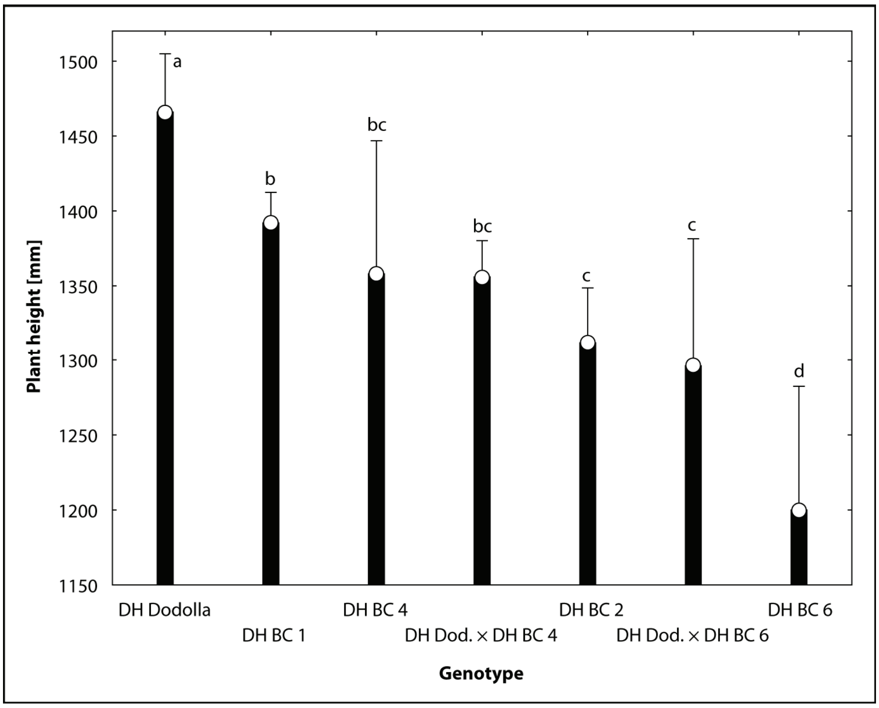
Blue – Populations, Green – DH lines, Red – Hybrids

Figure 2: Oil content in the seed of selected genotypes of *B. carinata* and *B. napus* planted in the glasshouse (in % of dry matter)



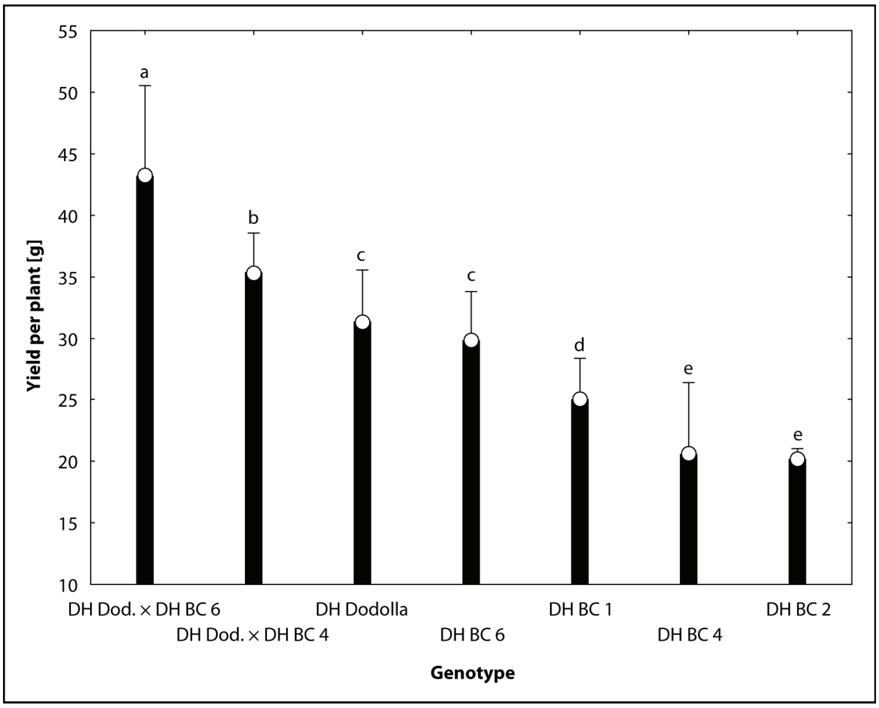
Blue – Populations, Green – DH lines, Red – Hybrids

Figure 3: Plant height in selected genotypes of *B. carinata* and their crosses; pooled data for five successive replications



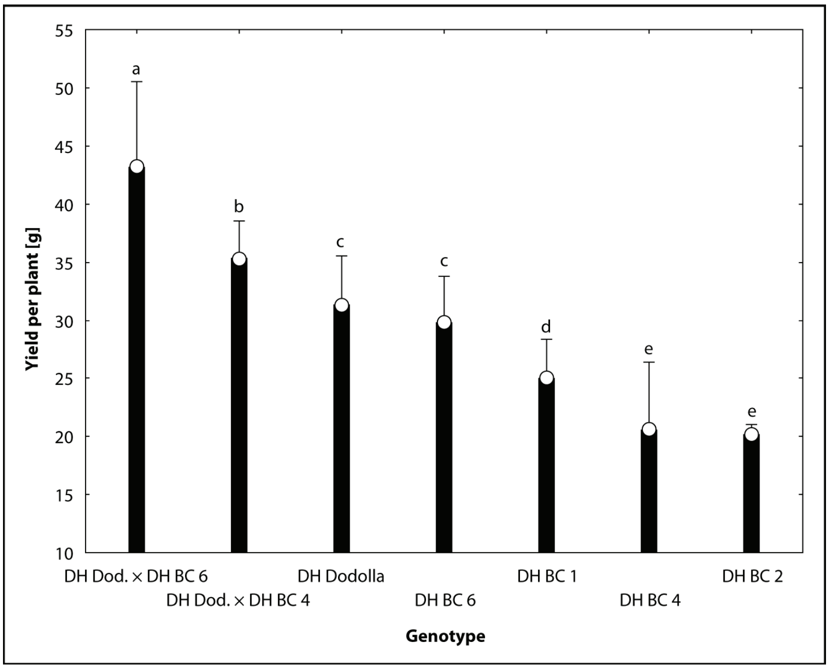
Bars represent individual 95% confidence intervals
Letters a-d designate homogeneous groups (LSD; $P = 0.05$)

Figure 4: Number of pods per plant in selected genotypes of *B. carinata* and their crosses; pooled data for three successive replications



Bars represent individual 95% confidence intervals
Letters a-d designate homogeneous groups (LSD; $P = 0.05$)

Figure 5: Seed yield per plant in selected genotypes of *B. carinata* and their crosses; pooled data for three successive replications

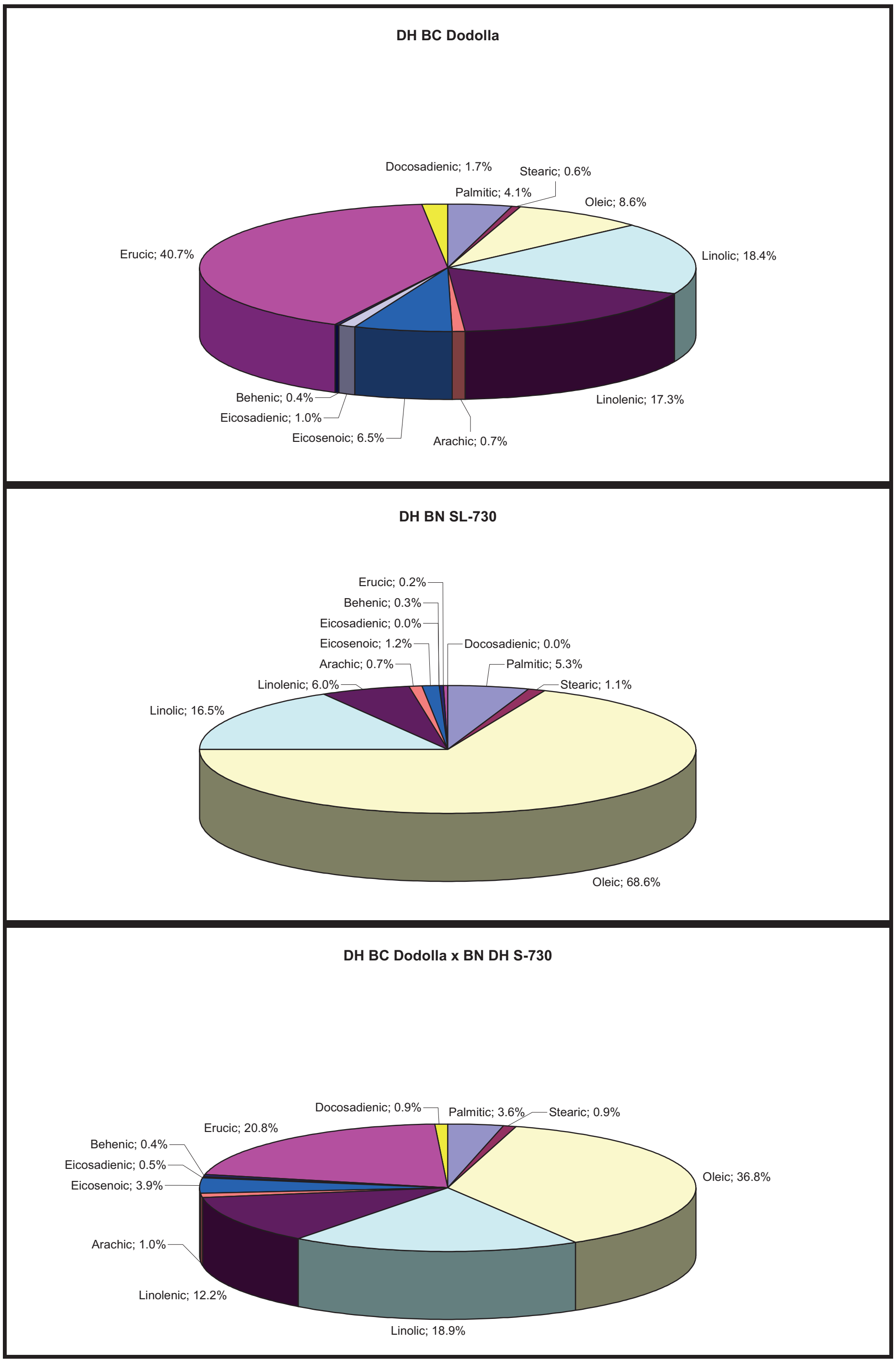


Bars represent individual 95% confidence intervals
Letters a-e designate homogeneous groups (LSD; $P = 0.05$)

Figure 6: Seed colour in selected genotypes of *B. carinata, B. napus* and their hybrids
Spot diameter = 60 mm

1 – BC 4
2 – DH BC Dodolla
3 – DH BC 4 x DH BC Dodolla
4 – (DH BC 4 x DH BC Dodolla) x DH BC Dodolla
5 – DH BN SL-730
6 – BC DH Dodolla x DH BN SL-730

Figure 7: Fatty acid composition of oil in the seed of DH lines and in F1 hybrid between *B. carinata* Dodolla and *B. napus* SL- 730



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